



Pollination ecology of *Bidens pilosa* L. (Asteraceae)

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ABSTRACT: Information on pollination ecology is essential to understand the sexual reproduction in *Bidens pilosa* L. The study is aimed at providing details of sexual system, breeding system, pollination mechanism, pollinators, seed dispersal modes and germination aspects *B. pilosa* based mostly on field study. Paper chromatography technique was used for recording sugar and amino acid types in the nectar since they are important to evaluate the pollination syndrome. The study indicates that *B. pilosa* displays vegetative, flowering and fruiting phases throughout the year. The plant produces heterogamous capitula with all ray florets opening on the first day and disc florets opening on the next four consecutive days. The ray florets are sterile while disc florets are fertile, dichogamous, protandrous, herkogamous, self-compatible, self-pollinating (vector-mediated) and facultative xenogamous. The disc florets display secondary pollen presentation. The tubate corolla, production of sucrose-rich nectar with essential and non-essential amino acids, and tri-colporate, echinate tri-colpate pollen grains in disc florets suggest entomophily. The plant is accordingly entomophilous but principally psychophilous. Disc florets produce non-dormant, long and short cypselas from the same capitulum. Seed dispersal is polychorous involving anemochory, anthropochory, zoochory and ombrohydrochory. The long cypselas disperse farther away from parental sites and germinate readily under a wide range of conditions while short cypselas disperse to short distances and germinate under specific germination conditions at parental sites/in similar habitats. Therefore, the plant with secondary pollen presentation, facultative xenogamy, insects as pollinators, bimorphic cypselas and polychory is able to grow as a widespread weed.

KEY WORDS: *Bidens pilosa*, Heterogamous head, Facultative xenogamy, Entomophily, Thripsophily, Bimorphic cypselas, polychory.

INTRODUCTION

Gillett (1975) reported that the genus *Bidens* is composed of approximately 230 species with worldwide distribution in tropical and temperate regions. Ballard (1986) and Jaimes and Ramirez (1999) noted that a few species of *Bidens* have been studied for their reproductive ecology in North and Central America. Grombone-Guaratini *et al.* (2004) reported that *Bidens pilosa* and *B. subalternans* in South America are protandrous, self-compatible and pollinated by hymenopterans and lepidopterans. Valentin-Silva *et al.* (2016) reported that *B. segetum* produces capitula with sterile ray florets and fertile hermaphrodite disk florets. Sun and Ganders (1988) reported that *Bidens* species in the Hawaiian Islands exhibit morphological and ecological diversity; their capitula produce sterile ray florets and fertile disc florets which are self-compatible and strongly protandrous. In these species, pollination is accomplished mainly by introduced honey bees. Grombone-Guaratini *et al.* (2004) reported that in *B. segetum* is herkogamous favouring xenogamy. Knight *et al.* (2005) reported that *B. segetum* is self-incompatible and principally butterfly-pollinated.

Sheriff (1937) classified *B. pilosa* into six varieties var. *pilosa*, var. *minor*, var. *radiata*, var. *bimucronata*, var. *calicicola*, and var. *alausensis* based on the

morphological differences. Peng *et al.* (1998) listed three varieties of *B. pilosa*, var. *minor*, var. *pilosa* and var. *radiata* in the Flora of Taiwan. Wu *et al.* (2004; 2010) reported that the most obvious differences in morphology among these three varieties are their flowers. The capitula of var. *pilosa* are discoid lacking ray florets, and the capitula of var. *minor* and var. *radiata* are radiate with ray florets. Of these, var. *radiata* is invasive while the other two varieties are non-invasive in Taiwan. Huang and Kao (2015) reported that *B. pilosa* var. *radiata* is a tetraploid, while var. *minor* and var. *pilosa* are hexaploids; var. *radiata* is self-incompatible, while the other two varieties are self-compatible and suggested that different ploid levels might result in different breeding systems among the three varieties. Ballard (1986) reported similar results for 200 populations of *B. pilosa* species complex in Central America, where the tetraploid populations are self-incompatible while the hexaploid populations are self-compatible. Huang and Kao (2015) reported that all the three varieties of *B. pilosa* are polyploids but ploid level is higher in non-invasive var. *minor* and var. *pilosa* than in invasive var. *radiata*. The var. *minor* and var. *pilosa* are more widespread and more dominant than var. *radiata* in the native distribution range of *B. pilosa* species complex. The studies mentioned above indicate that the detailed studies on the pollination ecology of *Bidens pilosa* have



not been done despite its widespread distribution in agricultural and non-agricultural areas. Keeping this state of information on this widespread species in view, the present study was contemplated to investigate the following objectives in *B. pilosa*: flowering phenology, flower morphology, floral biology, pollination mechanism, pollinators, sexual system and seed dispersal. This information is important to carry out further studies to develop strategies for its control in agricultural as well as non-agricultural areas if it is a menace.

MATERIALS AND METHODS

The study area: Populations of *Bidens pilosa* growing in Visakhapatnam region (17°42'N Longitude and 82°18'E Latitude) in Andhra Pradesh State, India were used for the study during 2015-2017. The climate is typically coastal, duly affected by the Northeast Monsoon (December-February) and the Southwest monsoon (June-September). The hot summer season is sandwiched between these two seasons. The months of October and November constituting "retreating monsoon" are cyclone prone and unpredictable weather prevails during this period. The temperature is the highest during May and then the weather is hot and humid and quite oppressive. Monsoon rains cool the tropical heat from June onwards. Since it is a coastal city, the maximum-minimum range is narrow and varies between 30°C and 42°C during day and between 20°C and 28°C during night. Rainfall ranges between 1000mm and 1500mm per annum.

Flowering phenology and morphology: Observations regarding the organization of inflorescences, the spatial positioning of flowers, and their position on the plant were made since these features are regarded as important for effecting pollination by foragers. Life time of individuals of two floret types was recorded by marking twenty just open florets each and following them until fall off. Anthesis was initially recorded by observing ten marked mature capitula in the field. Later, the observations were repeated five times on different days, each day observing twenty marked mature capitula in order to provide accurate anthesis schedule. Twenty mature disc florets were followed for recording the time of anther dehiscence. The presentation pattern of pollen was also investigated by recording how anthers dehisced and confirmed by observing the anthers under a 10x hand lens. The details of flower morphology such as flower sex, shape, size, colour, odour, sepals, petals, stamens and ovary were described.

Floral biology: Twenty mature but un-dehisced anthers from disc florets were collected from five randomly chosen plants and placed in a Petri dish. The pollen output per anther/disc floret and pollen-ovule

ratio was calculated using the protocol given by Cruden (1977). Individual volumes of nectar were recorded for twenty-five disc florets and then the average volume of nectar per disc floret was determined and expressed in μl . The capitula used for this purpose were bagged at mature bud stage, opened after anthesis and squeezed nectar from each disc floret into micropipette to measure the volume of nectar. Based on nectar volume in individual disc florets, the total volume of nectar secreted in a capitulum was estimated. Similarly, the nectar sugar concentration at capitulum level was determined using a Hand Sugar Refractometer (Erma, Japan). Nectar analysis for sugar types was done as per the Paper Chromatography method described in Dafni *et al.* (2005). The sugar content/flower is expressed as the product of nectar volume and sugar concentration per unit volume, mg/ μl . This is done by first noting the conversion value for the recorded sugar concentration on the refractometer scale and then by multiplying it with the volume of nectar/flower. Table 5.6 given in Dafni *et al.* (2005) was followed for recording the conversion value to mg of sugars present in one μl of nectar. Dinitrosalicylic acid method was followed for the first two sugar types while Resorcinol method for the last sugar type. The caloric reward of nectar/flower/day was measured as per the formula given in Heinrich (1975). He assumed that 1 mg of sugar yields 16.74 joules or 4 calories of energy and accordingly he used the formula for calculating the caloric reward of the nectar.

$$\frac{\text{Nectar volume } (\mu\text{l}) \times \text{Concentration of nectar } (\%) \times 16.74}{100}$$

Paper chromatography method described in Dafni *et al.* (2005) was followed for identifying the amino acid types in the nectar of disc florets.

Insect activity and pollination: The flower visitors were collected and identified with the representative specimens available with the Department of Environmental Sciences, Andhra University, Visakhapatnam. All butterflies were further confirmed by consulting the books of Kunte (2007) and Gunathilagaraj *et al.* (1998) while other insects, some to species level while a few others to genus level only. Thrips were identified using the key provided by Bhatti (1980) for Indian thrips. The insects were observed carefully for ten hours in a day for fifteen days in different months each year during profuse flowering period. The hourly foraging visits of each species were recorded on ten different days for which thirty capitula were selected. The data obtained was used to calculate the percentage of foraging visits made by each species per day and also to calculate the percentage of foraging visits of each category of insects per day. Simultaneously, the insects were observed for their foraging behavior such as mode of approach, landing,



Fig. 1. *Bidens pilosa*: a. Habit, b. Flowering phase, c. Flowering capitula, d. Capitulum with 5- ray florets, e. Capitulum with 7-ray florets.

probing behaviour, the type of forage they collected, contact with essential organs to result in pollination, inter-plant foraging activity. The insects were captured from the flowers during 1000-1200 h on five different days for pollen analysis in the laboratory. For each insect species, 10 specimens were captured and the proboscides were separated for examination to record whether they carry pollen grains or not. They were washed first in ethyl alcohol and the contents stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present.

Fructing ecology, seed dispersal and germination:

A sample of capitula was tagged and followed for fruit set rate of disc florets in open-pollinations and the same sample were used for noting the duration of fruit maturation. Seed characteristics of disc florets were carefully examined to note their special adaptations for dispersal mode. Field visits were made to record whether the seeds germinate immediately after they are dispersed or not. Field observations on seed germination and seedling formation were made to record the approximate number of generations produced during rainy season.

RESULTS

Phenology: The plant is an annual branching herb with gracile habit (Figure 1a). It grows in open hilly areas and waste lands but it is not very common. It often forms colonies covering the soil in open areas. The stems

are quadrangular with grooved and hairy straggling branches and striate branch lets. The leaves are petiolate, trifoliolate and arranged oppositely; terminal leaflets are obovate to lanceolate, crenate-serrate, acuminate, base rounded to acute and sparsely hairy. It propagates only by seed mode. The seeds germinate and produce new plants during rainy season which commences in June. The flowering occurs throughout the year if the soil is sufficiently wet but peak flowering occurs during September to November (Figure 1b, c). The plant withers and dies in areas where the soil is dry, especially during summer season.

Flower morphology: A plant produces 28.68 ± 17.42 heterogamous capitula either singly or in groups as a corymb on long peduncles in leaf axils or terminally. Each capitulum consists of a long outer and shorter inner linear to lanceolate involucre bracts with fine hairy margins followed by odorless ray and disc florets. In each capitulum, ray florets are usually five (Figure 1d) but rarely six or seven (Figure 1e) while disc florets are 34.68 ± 5.80 ; the ray and disc floret ratio is 1:6. The calyx is reduced to pappus of barbed and strong bristles in both types of florets. The corolla is tubate and tipped with five actinomorphic lobes in both floret types but in ray florets, one lobe is ligulate. The ligulate ray floret is white with scarlet red base which serves as nectar guide. The ray floret is 8.1 ± 1.1 mm long and 4.9 ± 0.6 mm wide. Disc florets are yellow, 8.5 ± 0.5 mm long and 1.8 ± 0.3 mm wide. The ray florets lack stamens but have



Fig. 2. *Bidens pilosa*: a. Anthesing capitulum, b-l: Disc florets - b-f. Different stages of anthesis, g. Position of stigma and anthers, h. Syngeneisous anthers, i. Pollen grain, j. Gynoecium, k. Ovary, l. Ovule.

poorly developed pistil with ovary lacking ovule and residual two-armed style. The disc florets have five stamens with yellow filaments (1.2 mm long and 1 mm wide) and dark brown anthers (1.8 mm long and 1 mm wide). The stamens are five, epipetalous and anthers are ditheous, fertile, united and cohered forming a hollow cylinder representing syngenesious condition (Figure 2h). The anther has its connective prolonged into a hood and tend to separate when the flower withers. The pistil is well developed with 2.5 mm long and 1 mm wide ovary with 5 mm long style which in turn is terminated into 2 stylar arms covered at the tip and the abaxial surface by sweeping hairs. A nectariferous disc is present at the base of the style inside the corolla tube. The style with its aligned arms extend beyond the height of anthers; the stylar arms diverge and curve inwards exposing the hidden stigmatic surfaces and finally over-arching the florets (Figure 2g). The ovary is bicarpellary, syncarpous and unilocular with one erect basal ovule (Figure 2j-l). The floral features indicate that ray florets are sterile female and disc florets are functionally bisexual.

Floral biology: The ray and disc florets open during early morning 0700-0900 h on clear sunny days (Table 1) (Figure 2a-f). The florets open completely on sunny days while they are partially open on rainy days. In a capitulum, all of the ray florets open on first day simultaneously; the disc florets open concentrically inwards from the next day for four successive days with 37% opening on day 1, 29% on day 2, 19% on day 3 and 15% on day 4. Individual disc

florets take about 3 hours to open from mature bud phase. The disc florets are protandrous with anther dehiscence taking place during mature bud stage by longitudinal slits. The narrow anthers are united to form a hollow space into which pollen is liberated. At mature bud stage the style with its aligned stylar arms lies below the anthers, its forked arms are converged at this stage. During and immediately after anthesis, the style grows, elongates and passes through the anther tube brushing the pollen with its sweeping hairs. At this stage, the inner stigmatic surfaces are un-receptive and not exposed, the functional situation of which prevents the occurrence of autogamy. Such a form of pollen presentation is referred to as "secondary pollen presentation mechanism" which ensures the pollen availability to insects visiting the capitula on daily basis. The style with its aligned branches gradually diverge in the early hours of 2nd day; then the inner stigmatic surfaces attain receptivity and remain so until the end of that day. The stylar arms curve downwards completely exposing the receptive stigmatic surfaces. The pollen grains are bright yellow, circular, tricolporate, $21.1 \pm 0.34 \mu\text{m}$ in size and echinate. The pollen grains are 338.21 ± 64.18 per anther, 1691.06 ± 320.94 per floret and 58,644 per capitulum. The pollen-ovule ratio is 1691:1. The ray florets are nectarless while disc florets are nectariferous. Ray florets do not produce nectar. Disc floret produces 1.2 μl of nectar which rises up as it accumulates in the floret due to narrow corolla tube by the end of 2nd day. Nectar is not secreted during the remaining period of floret life



indicating that nectar is secreted only during staminate phase. A capitulum produces an average volume of 41.6 μ l of nectar during its lifespan; the nectar sugar concentration is $25 \pm 1.43\%$ with 0.38 mg of sugar containing 1.52 calories of energy at floret level and 11.36 mg of sugar containing 45.44 calories of energy at capitulum level. The sugar types present in the nectar include sucrose, glucose and fructose; they are present in that order of dominance. The nectar contains four essential amino acids (tryptophan, histidine, lysine and arginine) and six non-essential amino acids (cysteine, serine, aspartic acid, glycine, cystine and glutamic acid); they are present in that order of dominance. The ray and disc florets wither away on 3rd day and fall off on 4th day. In fertilized disc florets, the tubate corolla and stamens gradually fall off after the initiation of fruit formation.

Table 1. Anthesis of disc florets as a function of time in *Bidens pilosa*

Time (h)	No. of disc florets anthesed								
	Day 1	%	Day 2	%	Day 3	%	Day 4	%	Total (%)
06:00	-	-	-	-	-	-	-	-	-
07:00	4	12	3	9	3	8	2	6	35
08:00	6	17	5	14	3	8	2	6	45
09:00	3	8	2	6	1	3	1	3	20

First, all ray florets open at once on the first day of anthesis of capitulum. The next day onwards disc florets open for four consecutive days. Total no. of disc florets per capitulum 35

Pollination mechanism: The disc florets present the stamens and stigmas at different positions. The anthers dehisce inwardly and discharge pollen grains into the anther tube during mature bud stage. At this stage, the style lies below the basal part of the anthers. During and immediately after anthesis, the style with its aligned stylar arms elongates within the anther tube and brushes the pollen out of the anther tube by stylar hairs called "sweeping hairs" presenting the pollen only on the outside of the style tip and stylar arms (sterile portions). Such a pollen presentation pattern is indicative of secondary pollen presentation functional through a combination of pump and brush mechanism. The stylar arms are in closed state on day 1 and the inside stigmatic surfaces are un-receptive, there is no possibility for the occurrence of autogamy. The staminate phase ends at 1700 h and the sterile portion of the stylar arms has residual pollen at that time. This is followed by the retraction of filaments and the consequent partial retraction of anthers into the corolla tube. The pistillate phase starts in the early hours of the 2nd day, the stigmatic surfaces attain receptivity, gradually diverge and curve downwards within three hours totally exposing the inner stigmatic surfaces; the stigmatic surfaces remain receptive until the end of that day. The secondary pollen presentation mechanism represented by a combination of pump and brush mechanism, and staminate phase on day 1 and pistillate phase on day 2 appear to have evolved to prevent autogamy and promote cross-pollination. However, the anthesis of disc florets for four consecutive days in the

same and different capitula on the same plant facilitates the occurrence of vector-mediated self-pollination. Therefore, the secondary pollen presentation mechanism and the sexual system functional do not insulate completely from the occurrence of self-pollination and hence the flowers set fruit and seed through self- as well as cross-pollination.

Thrips breeding, feeding and pollination: Thrips species, *Frankliniella schultzei* (Thysanoptera: Thripidae) oviposited during early bud stage of florets of capitula. The larvae emerge from the eggs in synchrony with anthesis and nectar production in disc florets. The centripetal development of the capitulum was found to provide continuous availability of pollen and nectar for four days for their growth. This species was found to feed on pollen and nectar from disc florets. Individual thrips were dusted with pollen in their upward and downward movements within the corolla tube of disc florets. The echinate pollen surface facilitated the thrips to carry 327 to 487 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on the stigmatic region of the stylar arms of disc florets due to their active movement, rubbing the abdomen on the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. Since the disc floret is staminate on day 1 and pistillate on day 2, the foraging activity of thrips within that floret does not contribute to self-pollination but may contribute to self-pollination between disc florets of the same or different capitula of the same plant. As the plant occurs as small or large populations, thrips could fly to migrate to the capitula of other closely spaced plants and effect cross-pollination.

Insect pollination: The capitulum is the unit of attraction for insect foragers. Within the capitulum, the white ligulate petals of sterile ray florets together with scarlet red nectar guide acts as chief attractant to direct the insect foragers to nectariferous disc florets to collect nectar and/or pollen. The disc florets were foraged by bees for nectar and pollen, and wasps, flies and butterflies for nectar. The butterflies were the consistent and regular foragers while all others were inconsistent foragers and also they forage especially during the peak flowering season. The bees were *Apis cerana* (Figure 3a), *Ceratina* sp. (Figure 3b) and *Andrena carantonica* (Figure 3c). The fly group was represented by bee-fly (unidentified) (Figure 3d). The wasp was *Campsomeris annulata* (Figure 3e). The butterflies included *Eurema hecabe* (Figure 3f), *Ypthima baldus* (Figure 3g), *Acrea violae*, *Neptis hylas* (Figure 3h), *Junonia lemonias*, *J. almana*, *Precis iphita* (Figure 3i), *Danaus chrysippus*, *Euploea core*, *Castalius rosimon*, *Caleta caleta* (Figure 3j), *Zizeeria karsandra* (Figure 3k), *Chilades pandava* (Lycaenidae), *Borbo cinnara* (Figure 3l), *Iambricx salsala* (Figure 3m) and *Nyctemera adversata* (Figure 3n) (Table 2). All these insects approached the flowers in upright position, landed on the flat-topped capitulum and

**Table 2.** List of insect foragers on *Bidens pilosa*.

Order	Family	Species	Common name	Forage sought
Hymenoptera	Apidae	<i>Apis cerana</i> F.	Indian Honey Bee	Pollen + Nectar
		<i>Ceratina</i> sp.	Small Carpenter Bee	Pollen + Nectar
	Andrenidae	<i>Andrena carantonica</i> Perez	Mining Bee	Pollen + Nectar
	Scoliidae	<i>Campsomeris annulata</i> F.	Sand Wasp	Nectar
Diptera	Bombyliidae	Unidentified	Bee fly	Nectar
Lepidoptera	Pieridae	<i>Eurema hecabe</i> L.	Common Grass Yellow	Nectar
	Nymphalidae	<i>Ypthima baldus</i> F.	Common Five Ring	Nectar
		<i>Acraea violae</i> F.	Tawny Coster	Nectar
		<i>Neptis hylas</i> Moore	Common Sailor	Nectar
		<i>Junonia lemonias</i> L.	Lemon Pansy	Nectar
		<i>Junonia almana</i> L.	Peacock Pansy	Nectar
		<i>Precis iphita</i> Cramer	Chocolate Pansy	Nectar
		<i>Danaus chrysippus</i> L.	Plain Tiger	Nectar
		<i>Euploea core</i> Cramer	Common Indian Crow	Nectar
	Lycaenidae	<i>Castalius rosimon</i> F.	Common Pierrot	Nectar
		<i>Caleta caleta</i> Hewitson	Angled Pierrot	Nectar
		<i>Zizeeria karsandra</i> Moore	Dark Grass Blue	Nectar
		<i>Chilades pandava</i> Horsfield	Plains Cupid	Nectar
	Hesperiidae	<i>Borbo cinnara</i> Wallace	Rice Swift	Nectar
		<i>Lambrix salsala</i> Moore	Chestnut Bob	Nectar
	Arctiidae	<i>Nyctemera adversata</i> Schaller	Tiger Moth	Nectar



Fig. 3. *Bidens pilosa*: a. *Apis cerana*, b. *Ceratina* sp., c. *Andrena carantonica*, d. Bee-fly (unidentified), e. *Campsomeris annulata*, f. Pierid, *Eurema hecabe*, g-i. Nymphalids – g. *Ypthima baldus*, h. *Neptis hylas*, i. *Precis iphita*, j. & k. Lycaenids – j. *Caleta caleta*, k. *Zizeeria karsandra*, l. & m. Hesperiiids – l. *Borbo cinnara*, m. *Lambrix salsala salsala*, n. Arctiid moth, *Nyctemera adversata*. o. & p. Cypsela dispersal (long and short), q. Short cypselas.

then probed disc florets for nectar. They foraged several disc florets in a single visit and made multiple visits to the few capitula produced by individual plants in quest of forage. They made frequent visits to capitula of different closely and distantly spaced plants to collect forage. Such a foraging behavior was considered to be promoting both self- and cross-pollination. The foraging

activity pattern of insects showed a definite pattern with reference to foraging schedule. They foraged flowers during 0800-1700 h with peak foraging during 1000-1200 h coinciding well with the standing crop of nectar by that time (Figure 4,5). Bees made 27%, wasps 6%, bee-fly 4% and butterflies 63% of total foraging visits (Figure 6). The body washings of insects collected from



Table 3. Pollen recorded in the body washings of insect foragers on *Bidens pilosa*.

Insect species	N	Number of pollen grains		
		Range	Mean	S.D
<i>Apis cerana</i>	10	65-218	139.7	36.56
<i>Ceratina</i> sp.	10	27-115	78.1	23.79
<i>Andrena carantonica</i>	10	53-144	102.6	26.39
<i>Campsomeris annulata</i>	10	20-39	28.4	5.66
Bee-fly (Unidentified)	10	13-34	23.5	6.67
<i>Eurema hecabe</i>	10	28-62	43.9	8.58
<i>Ypthima baldus</i>	10	16-41	31.5	6.48
<i>Acraea violae</i>	10	21-54	39.7	8.40
<i>Neptis hylas</i>	10	14-35	24.4	5.08
<i>Junonia lemonias</i>	10	22-51	36.1	7.10
<i>Junonia almana</i>	10	13-40	25.5	7.60
<i>Precisi phita</i>	10	18-43	33.8	6.32
<i>Danaus chrysippus</i>	10	12-37	27.6	6.44
<i>Euploea core</i>	10	20-49	38.2	7.78
<i>Castalius rosimon</i>	10	13-34	21.1	5.60
<i>Caleta caleta</i>	10	11-28	20.3	4.34
<i>Zizeeria karsandra</i>	10	9-24	17.8	5.45
<i>Chilades pandava</i>	10	12-30	20.4	4.62
<i>Borbo cinnara</i>	10	10-26	18.7	5.16
<i>Iambrix salsala</i>	10	7-22	19.4	4.52
<i>Nyctemera adversata</i>	10	11-28	19.8	6.05

(N: Sample size)

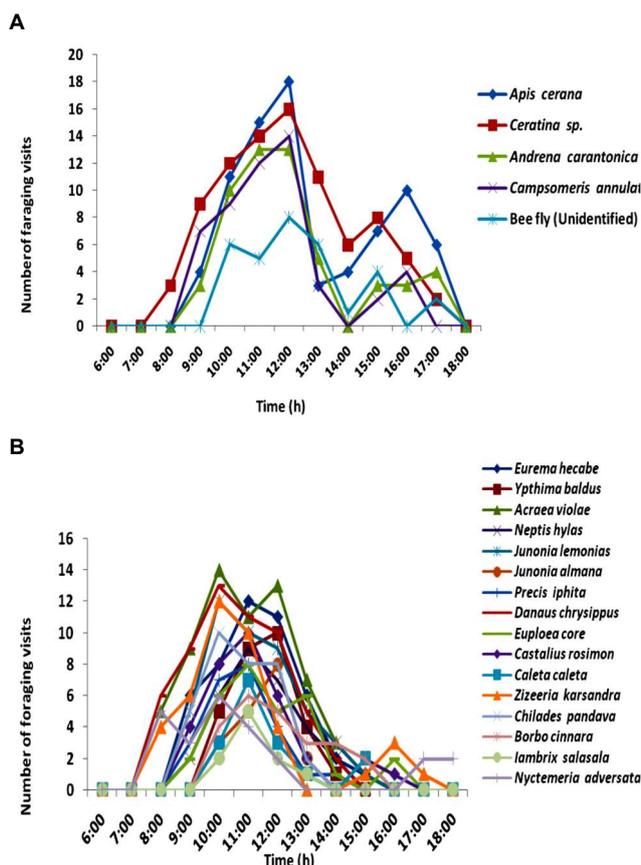


Fig. 4. Hourly foraging activity of (A) bees, wasps and bee-fly on *Bidens pilosa* (B) butterflies (day-long on a population of seventy-five flowering individuals)

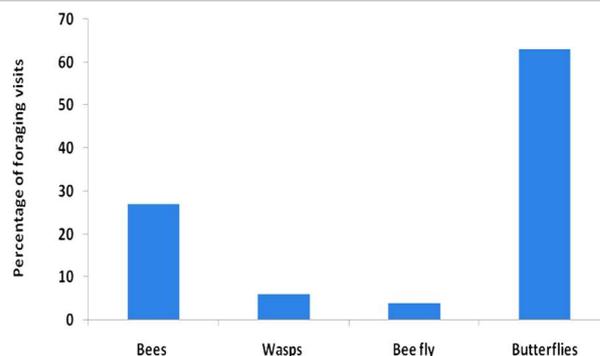


Fig. 6. Percentage of foraging visits of different categories of insects on *Bidens pilosa*.

the flowers during peak foraging period revealed that all insects carry pollen but bees carry the highest number of pollen grains. Further, the mean number of pollen grains varied with each insect species (Table 3).

Fruiting ecology and seed dispersal: The fertilized disc florets produce single-seeded cypsela within two weeks. Natural cypsela set is 84%. The cypsela is a brownish, 4-angled and sparsely hairy crowned with two or three barbellate awns. The cypsela morphology showed that there are two classes of cypselas, long cypsela (8-10 mm) without ornamentation of the tegument with thin seed coat and short cypsela (3-7 mm) with verrucose tegument and thick seed coat formed within each capitulum. The production rate of long cypsela is 64% and of short cypsela is 36%. The dry cypselas radiate in all directions from the capitulum base; the awns and verrucose tegument enable them to stick readily to hair, clothing and animal skin for dispersal (Figure 3o-q). Further, wind and water also disperse them. Therefore, anemochory, anthropochory, zoochory and ombrohydrochory are the modes of seed dispersal. Dry season and dry spells during rainy season favor the first three modes while rainy days favor the last mode of dispersal.

Field observations showed that long cypselas do not have dormancy and germinate readily within a week as soon as they are dispersed from the mother plants if soil is moderately wet while short cypselas have dormancy and germinate when seed coat degrades which usually occurs during rainy season. Long cypselas when sowed in pots filled with fertile soil germinated within a week and produced new plants subsequently while short cypselas when sowed in pots and watered regularly germinated only after 5 months. In natural areas, long cypselas produce three to four generations in a year depending on the soil moisture and nutrient status while short cypselas germinate in subsequent year(s) to produce new plants due to their dormancy. The long cypselas enable the weed to build up populations continuously while short cypselas enable the weed to keep them in reserve for population build up in specific environment.



DISCUSSION

Bidens pilosa is a therophyte herb and occupies roadsides, disturbed sites and cultivated fields in tropical and subtropical climates (Peng *et al.*, 1998; Grombone-Guaratini *et al.*, 2004). In India, it is widely distributed in both forest and cultivated sites. It produces three to four generations in moist soils and shows prolific growth by forming colonies. In such soils, it shows episodic vegetative, flowering and fruiting phases due to which it appears throughout the year. In other soils which are fed by only rain water, the plant shows prolific growth during the rainy season and the production of successive generations depends on the soil moisture content. It shows a peak flowering during September-November in rain-fed and irrigated soils. The plant disappears in moisture and nutrient-deficient soils, which is usually evidenced during dry season. Therefore, it can be said that soil moisture and nutrient content plays an important role in providing stimulus for the growth and reproduction of this plant.

Peng *et al.* (1998) reported *B. pilosa* has three varieties in Taiwan. They are var. *radiata*, var. *minor* and var. *pilosa*. The capitula in the first two varieties have both ray and disc florets while those in the last variety has only disc florets. Huang and Kao (2015) reported that *B. pilosa* var. *radiata* is a tetraploid and self-incompatible while var. *minor* and *pilosa* are hexaploids and self-compatible. Treier *et al.* (2009) reported that there is a link between polyploidy and invasiveness. All three varieties of *B. pilosa* are polyploids; var. *minor* and var. *pilosa* with hexploidy are non-invasive while var. *radiata* with tetraploidy is invasive in Taiwan. Further, these authors stated that non-invasive varieties of *B. pilosa* are more widespread and more dominant than invasive var. *radiata* in the native distribution of *B. pilosa* species complex. Keeping this in view, they stated that polyploidy alone cannot explain the dominance of non-invasive varieties and suggested that there are some other traits such as the ability of vegetative reproduction and breeding system that contribute to dominance or invasiveness of *B. pilosa* varieties. In the present study, the distribution and floral morphological characters of *B. pilosa* probably indicate that this weed represents var. *pilosa* which is dominant in cultivated and forest sites with moist soils but it is not invasive. However, further studies on vegetative and reproductive aspects are needed for confirmation.

Dichogamous protandry and self-incompatibility have been reported to be functional in Asteraceae (Allen *et al.*, 2011). Dichogamous protandry is a mechanism that reduces the impact of pollen-pistil interference on pollen import and export (Barrett, 2002) and reduces rates of self-fertilization and enhances out-crossing (Harder *et al.*, 2000). Self-incompatibility is another evolutionary strategy to avoid self-fertilization and

inbreeding (Nettancourt, 2001). In Asteraceae, it usually occurs at the stigmatic surface either by failure of germination of self-pollen grains or by the arrest of pollen tube growth (Allen *et al.*, 2011). The present study shows that *B. pilosa* exhibits dichogamous protandry and self-compatibility. The ray florets representing female sex are sterile due to non-production of ovule by ovary. The disc florets are bisexual and fertile with staminate phase on day 1 and pistillate phase on day 2 characterize dichogamous protandry characterizing temporal dioecy. The florets also have a physical barrier between the site of pollen deposition and stigmatic surfaces characterizing herkogamy which favors xenogamy, and pollination occurs during pistillate phase. The vector-mediated selfing occurs due to availability of self-pollen as a consequence of centripetal opening of disc florets in the capitulum on consecutive days. Such a mating system has been reported in *Bidens segetum* (Grombone-Guaratini *et al.*, 2004). In this study, high fruit set rate within the capitulum and in open-pollinations at population level in *B. pilosa* indicate the function of self-compatibility and self-pollination in this plant. This finding agrees with the report of Huang and Kao (2014) that hermaphrodite disc florets of *B. pilosa* var. *pilosa* are self-compatible, self-pollinating and set fruit in bagged capitula in Taiwan, and also with the report of Grombone-Guaratini *et al.* (2004) that *B. pilosa* is self-compatible and self-pollinating. The pollen/ovule ratio recorded for *B. pilosa* (1,691:1) in this study falls in the range of pollen/ovule ratio (244.7-2,588) for facultative xenogamy provided by Cruden (1977) and hence this plant is facultative xenogamous with low self-incompatibility. In this breeding system, xenogamy enables the plant to increase genetic heterogeneity which favors its establishment in heterogeneous and variable environments (Hsu, 2006) while geitonogamy facilitates to increase its population in the currently growing sites or other sites with similar environment. The allied species, *B. alba* and *B. subalternans* are also self-compatible and self-pollinating (Grombone-Guaratini *et al.*, 2004). But, a congener, *B. segetum* is self-incompatible as it does not set fruit in spontaneous and hand-self pollination modes (Valentin-Silva *et al.*, 2016). Therefore, it can be stated that *Bidens* genus has both self-compatible and self-incompatible species with which they became widespread while some of them became invasive.

In Asteraceae, secondary pollen presentation mechanism is an important characteristic associated with protandry (Howell *et al.*, 1993) but it is an ancestral feature in this family (Jeffrey, 2009). This pollen presentation mechanism has been considered to be a strategy to improve accuracy in pollen removal and deposition in order to enhance male and female fitness (Ladd, 1994). In the present study, the disc florets of *B. pilosa* are protandrous and the pollen shed from the



anthers is brushed by the sterile sweeping hairs present at the tip and abaxial side of stylar arms when the style branches are joined and inner stigmatic surfaces are not receptive. This pollen presentation mechanism combined a pump and a brush mechanism which is a typical feature in the Asterales complex (Leins and Erbar, 2006). As the style grows out of the anther tube, the outer sweeping hairs of the style arms present pollen for pollination. The receptive papillate stigmatic surface is hidden between the two appressed style arms, preventing self pollination. During the pistillate phase, the style arms separate exposing the receptive papillae for the receipt of pollen. The style arms serve as secondary pollen presenters in the staminate phase and expose receptive stigmatic surfaces for pollen during pistillate phase. This type of active pollen presentation is typical of disc florets of Asteraceae (Ladd, 1994). The secondary pollen presentation system functional in *B. pilosa* appears to have evolved to enhance the efficiency and accuracy of pollen exportation and/or pollen reception, thus increasing male and/or female fitness of the plant (Yeo, 1993).

In *B. pilosa*, the capitulum with peripheral nectarless ray florets attract foragers. In the central disc florets, narrow tubular corolla, nectar production, pollen accessibility to foragers within and outside the corolla tube, and pollen characteristics such as circular shape, tri-colporate apertures and echinate exine are adaptations for insect-pollination. Wodehouse (1935) stated that echinate character of the pollen grains is a special adaptation for adherence to insect vectors. Therefore, the characteristics of disc florets suggest that this plant is adapted for insect-pollination.

Baker and Baker (1982; 1983) reported two categories of flowers "true butterfly flowers" and "bee and butterfly flowers" based on flower-butterfly relationships. In *B. pilosa*, the floret characteristics such as ray florets with nectar guide but without nectar, disc florets without nectar guide but with minute volume of sucrose-rich nectar with moderate sugar concentration and negligible level of sugar content, and some essential and non-essential amino acids at the base of the narrow corolla tube conform to "true butterfly pollination syndrome".

In *B. pilosa*, the capitula attract pierid, nymphalid, lycaenid, hesperiid and arctiid butterflies but the nymphalids and lycaenids are principal pollinators as they are more speciose than other families of butterflies. It is interesting to note that papilionid butterflies never visited the capitula. The small, narrow tubate disc florets aggregated into capitula are appropriate for pollination by butterflies which efficiently handle several florets to collect nectar successfully in a single visit. In such an act, their proboscis gains contact with the dehisced anthers situated inside the corolla tube and also with the pollen adhered to sweeping hairs. The proboscis is the carrier

of pollen and the number of pollen grains carried by them vary with the length of proboscis and the time of nectar collection. In pistillate phase, the butterflies with their proboscis contact the exposed stigmatic regions and in effect transfer and deposit pollen at this region effecting pollination. Self-pollination occurs due to the occurrence of staminate and pistillate phase of florets at capitula and plant level. The standing crop of nectar at plant or population level is commensurate with the requirement of the butterflies as sufficient volume of nectar is available at capitulum level. The butterflies frequent the capitula of the same and/or different closely or distantly spaced individuals and promote cross-pollination. Their foraging schedule and the peak foraging activity period coincide well with the availability levels of standing crop of nectar. The consistent foraging activity of butterflies evidenced on *B. pilosa* suggests that they use this plant as a major nectar source. Therefore, *B. pilosa* is principally psychophilous.

In *B. pilosa*, bees, wasps and bee-fly also use the florets as pollen and/or nectar sources. The foraging behavior of the bees indicated that they are successful mostly as pollen collectors due to a slight mismatch between the length of their tongue and the length of the corolla tube to collect the deeply seated nectar. In case of wasps and bee-fly, their proboscis length easily facilitates nectar collection from the disc florets. All these insects carry pollen on their body, transfer and effect self- and cross-pollination. Since they are inconsistent foragers and also they are almost confined to peak foraging activity, they qualify as supplementary pollinators. Further, the thrips species, *Frankliniella schultzei* uses this plant as breeding and feeding sites. The larvae and adults of this thrips emerge during anthesis from mature buds, move in and out of the florets collecting pollen and nectar from disc florets due to which self-pollination occurs. Their role in pollination is substantiated by their efficient pollen carry-over capacity. As there is a continuous emergence of thrips due to centripetal anthesis in disc florets, the available nectar crop becomes insufficient for them and they then migrate to the capitula of the same and different plants and effect cross-pollination. Apart from this, their nectar feeding activity reduces the availability of nectar at capitulum level and drive all visiting insects to make several visits to disc florets of capitula of different plants for forage. Therefore, thrips indirectly promote out-crossing by all insects.

Mani and Saravanan (1999) stated that butterflies are important pollinators of Asteraceae and represent nearly 75% of all visitors. Because their irregular, unpredictable and random foraging behavior increases out-crossing rate which in turn increases genetic variation. These authors also stated that Syrphidae flies are also important pollinators. Sun and Ganders (1990) reported that butterflies are important foragers of *Bidens*



species. Grombone-Guaratini *et al.* (2004) reported that *Bidens pilosa* var. *pilosa* is principally pollinated by bees, especially solitary bees which tend to visit the capitula randomly promoting out-crossing while butterflies are of minor importance as they do not carry pollen on their bodies. The present study shows that butterflies are principal pollinators while all other insects are supplementary pollinators of *B. pilosa*.

Herrera (2009) stated that seed dispersal is mainly determined by fruit characteristics, which usually vary continuously within an individual plant. Imbert (2002) stated that some plants are heterocarpic producing two or more defined fruit types with distinct fruit morphs that may show different behavior for dispersal, germination recruitment or seedling survival. Harper (1977) stated that Asteraceae members are well known for their polymorphic fruits and their capitulum structure allows morphological variation in the fruits produced. Torices *et al.* (2013) reported that in spite of the high incidence of heteromorphy in this family and its known influence on dispersal ability and germination performance, the proximate causes of cypselas variation remain unknown. The fruit is usually referred to as "seed" in this family and it is called as cypselas or achene. Corkidi *et al.* (1991) reported that *Bidens odorata* and *B. gardneri* produce polymorphic cypselas. Forsyth and Brown (1982) reported that *B. pilosa* produces dimorphic cypselas, long and short. Amaral and Takaki (1998) reported that the production of long and short cypselas in *B. pilosa* depends on the age of the plant but they have not reported whether this plant produces both types of cypselas within the same capitulum or in different capitula. The present study reports that *B. pilosa* produces bimorphic cypselas (long and short) within the same capitulum and also their production is not associated with the age of the plant. The long cypselas lack ornamentation on the tegument while short cypselas contain verrucose ornamentation on the tegument. Amaral and Takaki (1998) also reported similarly regarding the ornamentation of the teguments in the cypselas of *B. pilosa*. In the present study, the long cypselas contain thin coat and light weight when compared to short cypselas in *B. pilosa*. In effect, long cypselas detach easily from the dry capitula and disperse long distances (anemochory) while short cypselas either detach from the dry capitula and disperse short distances (anemochory) or remain so within the capitula until the rainy season, and then sequentially detach during different periods of rain (ombrohydrochory). Ombrohydrochory may be advantageous in fluctuating environments because it increases the probability of establishment during different pulses of precipitation when the likelihood of establishment success is maximal. Further, both types of cypselas disperse through humans and animals by sticking to their bodies with their awns. Therefore, cypselas dispersal in *B. pilosa* is polychorous involving anemochory, ombrohydrochory, anthropochory and zoochory.

In the Asteraceae member, *Anacyclus clavatus*, larger cypselas show a higher probability of germination than those of smaller ones (Rai and Tripathi, 1982; Pandey and Dubey, 1988), regardless of the cypselas dispersal ability (McEvoy, 1984). Such differences in germination have been mainly attributed to seed size rather than to differences in cypselas morphology (Van Molken *et al.*, 2005). In *B. pilosa*, long cypselas do not have dormancy while short cypselas have dormancy. Long cypselas germinate readily under a wide range of conditions while short cypselas germinate only when specific germination conditions exist. Short cypselas have thick seed coat compared to the seed coat of the long cypselas. The thin coat of the long cypselas may imply ease of imbibition, of passage of oxygen to the embryo and of rupture of the coat by the radicle. In keeping with the opportunistic requirements of weeds in general, the long cypselas of *B. pilosa* show no stringent requirements and thus many seeds may be sacrificed if unfavorable conditions prevail in the area to which seeds are dispersed. The short cypselas with their more demanding germination requirements may represent a back-up population germinating only under conditions more favorable for subsequent growth of seedlings. The short cypselas are the heavier of the two seed types and this may result in their dispersal in the immediate vicinity of the parental plant. Their thicker seed coats imply that a period of burial in the soil may be required to degrade the coat. Once buried their germination would be prevented by a requirement for light. Therefore, short cypselas germinate only when assured favorable conditions exist and ensure the plant to maintain the widespread status as a weed. Further, the heterocarpy represented by bimorphic cypselas is a mixed strategy in which seed subsets of one individual may successfully germinate under different conditions in time and space. This offers the chance to colonize new sites, free from sibling competition or other local sources of stress, whereas the remainder of the offspring stays in the same habitat (Imbert and Ronce, 2001). The study suggests that *B. pilosa* with bimorphic cypselas and the associated traits is able to grow as a widespread weed in different habitats and expand its distribution range. This plant is considered to be a menace in agro-ecosystems. But it is an important food source for bees, wasps, flies and butterflies. Grombone-Guaratini *et al.* (2004) also reported that *B. pilosa* is an important food source for hymenoptera and diptera. Therefore, it may be an important component of agro-ecosystems because it plays a significant role in sustaining communities of insects that contribute to crop growth.

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